AVIAN CHEMICAL REPELLENCY: A STRUCTURE-ACTIVITY APPROACH AND

IMPLICATIONS

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INTRODUCTION

Until recently, the discovery of avian sensory repellents has been empirical (Mason, Adams and Clark 1989). However, recent studies in our laboratory have shown that many avian repellents have similar perceptual and structural properties (Mason et al. 1989; Mason Clark and Shah 1991; Clark and Shah 1991; Clark, Shah and Mason 1991; Shah, Clark and Mason 1991). For example, methyl anthranilate, which has a grapy odor, is repellent to birds (Kare and Pick, 1960). Ortho-aminoacetophenone has an odor and structure similar to that of methyl anthranilate, differing only in the substitution of a ketone for an ester group (Mason et al. 1991). Behavioral tests of this aminoacetophenone isomer showed that it is at least an order of magnitude more repellent to birds than methyl anthranilate (Clark and Shah 1991). These similarities in structure and function prompted us to undertake a series of studies to elucidate a predictive model of chemical structure-activity (Clark and Shah 1991; Clark et al 1991; Shah et al. 1991). As a consequence of these studies we hypothesize a model where the following structural features appear to be important:

(1) A phenyl ring with an electron donating or a basic group is central to repellency;

(2) An electron withdrawing group in resonance with a basic group decreases the repellency (as well as the toxicity) of a substance. These effects are pronounced when the groups are ortho to one another;

(3) The presence of an acidic group decreases repellency;

(4) The presence of an H-bonded ring or a covalently bonded fused ring that possesses the required features (e.g., electron donating and withdrawing groups ortho to each other) can enhance repellency, but is not essential;

(5) Steric hinderance can overpower the features described above, and can weaken the effectiveness of potentially aversive substances.

This model was arrived at by examining analogues of benzoic acid, its esters (anthranilates) and acetophenones from various perspectives. In particular, we studied (a) the relationship of repellency to H-bonded rings fused or adjacent to the phenyl ring; (b) the effect of increased or decreased electron donation on the phenyl ring; (c) the effect of increased or decreased electron withdrawal; (d) the effects of a fused heterocyclic ring that incorporates polar groups; (e) the importance of steric effects; and (f) the importance of the phenyl ring, per se. Our studies were thus designed to elaborate the steric and electronic factors involved in behavioral aversiveness from a chemical point-of-view. The data do not address biochemical and/or physiological pathways that could mediate responding.

CHEMICAL PARAMETERS OF BEHAVIORAL AVOIDANCE

Relationship of repellency to an H-bonded ring fused or adjacent to the phenyl ring

We studied the importance of this parameter by examining positional isomers of aminoacetophenones [i.e., 2 (ortho), 3 (meta), 4 (para)] as well as alpha-aminoacetophenone (here, the amino group is on the side chain) (Figure 1). Our data showed that the ortho-isomer was more active than the para-isomer, and that the para-isomer was more active than the meta-isomer. Alpha-aminoacetophenone repellency was intermediate to ortho- and para-aminoacetophenone (Mason et al. 1991). Examination of methyl anthranilate analogues showed a similar trend (Clark et al. 1991). To further explore the possible role of the H-bonded ring, we next substituted the amino group with hydroxyl and methoxyl groups. Although slightly less active than the amino derivatives, the methoxy derivatives were superior when compared to their hydroxy counterparts (Clark and Shah 1991). Here too the 2-substituted isomers were more active.
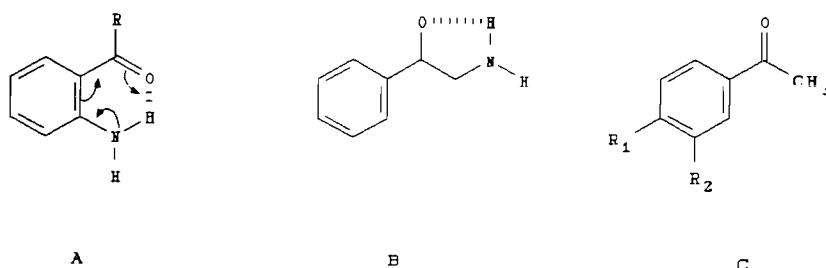

Figure 1. Positional isomers of aminoacetophenones. The arrows indicate electron withdrawal and donation paths. (A) When R = CH3, orthoaminoaceto-phenone; R = OCH3, methyl anthranilate. (B) Alpha-aminoacetophenone. (C) R1 = H and R2 = NH2, 3-aminoacetophenone; when R1 = NH2 and R2 = H, 4-aminoacetophenone.

Effect of increased or decreased electron density on the phenyl ring

Increased electron density on the phenyl ring can be obtained in two ways, namely through the addition of electron donating groups or by decreasing electron withdrawal. Conversely, electron density can be diminished by the addition of electron withdrawing groups or the removal of electron donating groups. Methyl groups donate electrons via inductive effect. When N-methyl-methylanthranilate (dimethyl anthranilate) was tested (Clark et al. 1991), it was slightly more repellent than methyl anthranilate. This finding,
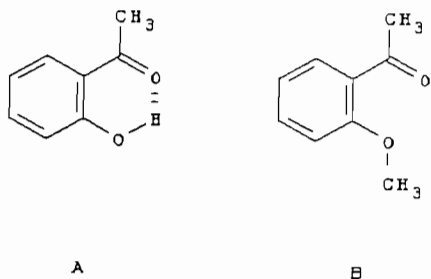
Figure 2. (A) 2-hydroxyacetophenone. (B) 2-methoxyacetophenone.

If an H-bonded ring (i.e., an intramolecular H-bond) was an essential feature for repellency, then hydroxy derivatives would have been more effective than their methoxy counterparts (Figure 2). Since this result was not obtained, we examined the role played by the basic nature of the substituent groups.

together with the data for methoxy and hydroxy aminoacetophenone derivatives, showed that electron donation (or basicity) of the substituent was important. But still, the meta isomers were almost inactive, suggesting that the resonance of this basic group with the carbonyl or carboxyl moiety was affecting repellency. Also, the ortho-isomer was still superior to the para-isomer, suggesting a possible ancillary role played by the H-bonded ring. When electron donation by the amino group, was compensated by a nitro group, in resonance (Clark et al. 1991), the compound (2-amino-5-nitro benzoic acid) was not repellent to birds.

Similarly, when the electron withdrawing sulfonyl group was placed between the amino group and the phenyl ring, as in the case of orthocarbo-ethoxy-benzene sulfonamide, no aversiveness was observed (Clark et al. 1991). Alternatively, when 2'-amino-4',5'-dimethoxy-acetophenone was examined, no substantial increase in the repellency was observed. Thus, increased electron richness by dimethoxy substitution did not influence repellency. However, increased electron withdrawal decreased aversiveness. Together, these results suggest an upper limit to enhanced repellency associated with electron enrichment of the phenyl ring. When the electron donating group was removed as in the case of acetophenone or methyl benzo-ate, the compounds were found to be ineffective (Clark et al. 1991; Shah et al. 1991). When an amino group was reintroduced at a different place (away from the phenyl ring), as in alpha-aminoacetophenone, repellency was partially restored (Mason et al. 1991). This reintroduction of the amino group also had a limited effect since benzamide was ineffective as a repellent although theoretically, the amino group in that position should have compensated the electron withdrawal by the carbonyl group.

Interestingly, when the electron withdrawing carbonyl group was removed from the phenyl ring, toxicity was observed. Tests of 2-amino-benzyl alcohol showed that the compound was moderately repellent at low concentrations, but lethal at higher levels. Thus the electron withdrawing group may be associated with decreased repellency, as well as decreased toxicity.

Effect of fused heterocyclic rings

Since the fused H-bonded ring structure was found to play at least an ancillary role, it was of interest to find the effect of covalently bonded rings. Two compounds with covalently bonded rings were initially tried,

viz. isatoic anhydride and 4-ketobenztriazene (Clark et al. 1991). Both of these substances have the required electron donating amino and electron withdrawing carbonyl functionalities ortho with respect to each other (Figure 3). The difference between the two lies in the interlinking atoms that make the covalently bonded heterocyclic ring. In the case of isatoic anhydride, the amino group is connected to a carbonyl group and thus reduces the electron donation to the phenyl ring, whereas in 4-ketobenztriazene the amino group is connected to the carbonyl group via two nitrogen atoms. Apart from keeping the possible electron donation by the amino group the same, the nitrogen atom at position 3 can potentially reduce the electron withdrawal by the carbonyl group via internal compensation. As predicted, isatoic anhydride was found to be ineffective and 4-ketobenztriazene was as active as ortho-aminoacetophenone.

Anthraquinone has been registered as an effective avian repellent. If this substance was truly effective, then the result would contradict our understanding of avian repellency. Anthraquinone contains two electron withdrawing carbonyl groups ortho to each other, linked via two carbon atoms which form a part of another phenyl ring. When we evaluated the repellency of anthraquinone, however, we found it to be entirely ineffective. The fact that a registered bird repellent would be ineffective is not surprising (e.g., Dolbeer, Link and Woronecki 1988). However, we deduce that although the effectiveness of a stimulus cannot be increased (compared to orthoaminoacetophenone) by a covalently bonded fused ring structure, it can be decreased by placing electron withdrawing groups in the fused ring.

Steric effects

Steric effects were studied using a variety of anthranilate derivatives (4). We observed that increasing the bulk on either the amino group or the ester group decreased repellency if the group involved could potentially decrease the pi-orbital overlap by forcing them partially out of plane. Thus N,N-diisobutyl-methyl anthranilate, as well as phenethyl anthranilate, were ineffective repellents, whereas methyl anthranilate, dimethyl anthranilate, and ethyl anthranilate showed good activity. One compound in this series, namely linalyl anthranilate, showed good repellency, although the group itself was quite large. This finding might be due to planar positioning of the initial sp2 hybridized atoms of the linalyl group which would keep its chain away from the amino group.
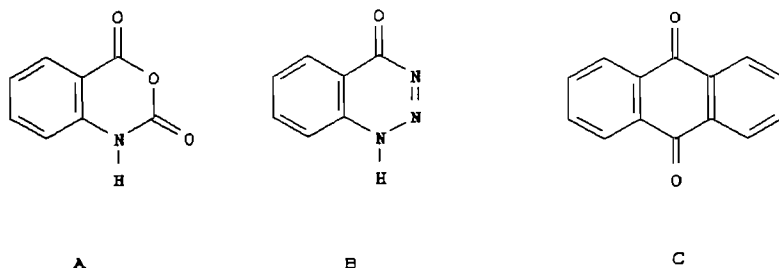

Figure 3. Structures for (A) isatoic anhydride, (B) 4-ketobenztriazine, (C) anthraquinone.

The importance of the phenyl ring

When the phenyl ring from methyl anthranilate is removed, the result-
ing compound is an ester derivative of beta-alanine. Hence, we tried
beta-alanine itself as a potential repellent. It had no apparent aversive
quality. These data suggest two possibilities. The first possibility is
that a phenyl ring may be required for repellency. Alternatively, it may
be that the position of the two polar groups must be rigid. In the latter
case, no phenyl ring need be present. We are currently exploring these two
possibilities.

ON-GOING RESEARCH

We are applying our model to the GRAS list of substances that are
approved by the U.S. Food and Drug Administration for human consumption.
First, this provides opportunities to evaluate the predictive capacities of
our model. Also, from a practical perspective, our expectation is that
substances will be more easily registered by the U.S. Environmental Protec-
tion Agency as avian repellents if they are already approved for use in
human and/or animal feeds. To this end, we tested vanillin, a widely used
flavoring agent, and two derivatives, vanillyl alcohol and veratryl alcohol
(5). These substances were chosen as stimuli because they permitted us to
examine the roles played by electron donating and withdrawing (polar)
groups and their contribution to resonance in the absence of a confounding
H-bonded ring at the ortho position (Figure 4).

In vanillin, the electron-withdrawing group is in resonance with the
hydroxyl group. This hydroxyl group and the methoxyl group form a 5-mem-
bered H-bonded ring. Vanillyl alcohol entirely eliminates the electron
withdrawal by resonance. Thus, comparison of data for vanillin and vanil-
lyl alcohol would show the importance of the electron-withdrawing group.
Examination of veratryl alcohol would show the role of electron-donating
groups and the importance of the H-bonded ring for repellency because it
has neither an electron-withdrawing carbonyl group, nor an H-bonded ring.
Instead, it has a methoxyl group (rather than hydroxyl group), resulting in
increased electron-donation to the phenyl ring. Overall, then, we predict-
ed that veratryl alcohol should be a more effective repellent than either
vanillyl alcohol or vanillin. The results showed that veratryl alcohol was
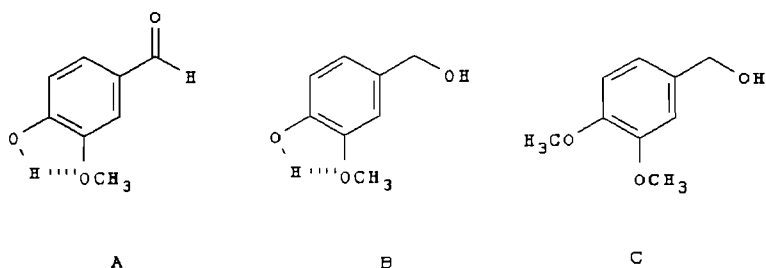significantly more repellent (Shah et al. 1991).

Figure 4. Structures for (A) vanillin, (B) vanillyl alcohol, (C) veratryl
alcohol.

We also are applying our model to GRAS listed substances such as
derivatives and analogs of coniferyl benzoate, a naturally produced materi-
al known to deter the feeding activity of ruffed grouse (Bonasa umbellus)
and other birds (Jakubas, Mason Shah and Norman 1991). Coniferyl benzoate

may be regarded as a derivative of cinnamyl alcohol. Although these compounds are very dissimilar to the compounds described above (Figure 5), we speculated that the electronic effects could still be used to predict repellency. Seven different compounds were tested in this series of experiments. The results obtained were broadly consistent with our hypothesis: dimethoxy cinnamyl alcohol and its benzoate were the best stimuli whereas coniferyl alcohol and its benzoate were the worst, showing the negative effects of the phenolic hydroxyl group.
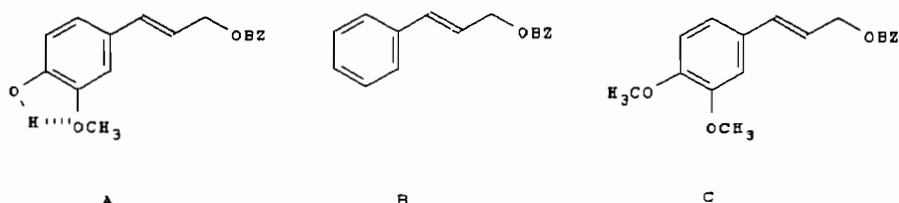

Figure 5. Structures for (A) coniferyl benzoate, (B) cinnamyl benzoate, (C) 3,4-dimethoxy cinnamyl benzoate.

CONCLUSIONS

From our experiments, it appears that potential repellents can be predicted on the basis of a few molecular attributes. Although this model provides a good qualitative correlation of aversiveness to basic chemical structure, it is unable to predict aversiveness in quantitative terms, e.g., shape of dose-response curves. For this reason, we have begun to use molecular modelling to examine the structure of compounds more closely. Also, we are testing different types of chemical substances to evaluate and refine the accuracy of the present model.

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